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Review

Effects of thermal food processing on the chemical structure and toxicity of fumonisin mycotoxins

Hans-Ulrich Humpf[1] and Kenneth A. Voss[2]

[1]Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster, Münster, Germany
[2]U.S. Department of Agriculture, Agricultural Research Service, Toxicology and Mycotoxin Research Unit, Richard B. Russell Agricultural Research Center, Athens, GA, USA

Fumonisins are *Fusarium* mycotoxins that occur in corn and corn-based foods. They are toxic to animals and at least one analogue, fumonisin B_1, is carcinogenic to rodents. Their effect on human health is unclear, however, fumonisins are considered to be risk factors for cancer and possibly neural tube defects in some heavily exposed populations. It is therefore important to minimize exposures in these populations. Cleaning corn to remove damaged or moldy kernels reduces fumonisins in foods while milling increases their concentration in some and reduces their concentration in other products. Fumonisins are water-soluble and nixtamalization (cooking in alkaline water) lowers the fumonisin content of food products if the cooking liquid is discarded. Baking, frying, and extrusion cooking of corn at high temperatures (≥190°C) also reduces fumonisin concentrations in foods, with the amount of reduction achieved depending on cooking time, temperature, recipe, and other factors. However, the chemical fate of fumonisins in baked, fried, and extruded foods is not well understood and it is not known if the reduced concentrations result from thermal decomposition of fumonisins or from their binding to proteins, sugars or other compounds in food matrices. These possibilities might or might not be beneficial depending upon the bioavailability and inherent toxicity of decomposition products or the degree to which bound fumonisins are released in the gastrointestinal tract. In this review the affects of cooking and processing on the concentration and chemical structure of fumonisins as well as the toxicological consequences of known and likely fumonisin reaction products are discussed.

Keywords: Corn / Fumonisin mycotoxins / Liquid chromatography – electrospray ionization – tandem mass spectrometry / Review / Thermal food processing

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Contents

Correspondence: Dr. Hans-Ulrich Humpf, Institut für Lebensmittelchemie, Westfälische Wilhelms-Universität Münster, Corrensstr. 45, D-48149 Münster, Germany
E-mail: humpf@uni-muenster.de
Fax: +49-251-83-33396

Abbreviations: **FB_x**, fumonisins; **TCA**, propane-1,2,3-tricarboxylic acid

1 Introduction

The fumonisins are a group of structurally related metabolites of *Fusarium verticillioides* (formerly *Fusarium moniliforme*) [1], one of the most common field fungi associated with corn and corn-based foods and feeds world-wide [2]. Besides *F. verticillioides*, other *Fusarium* species such as *F. proliferatum* are also important mycotoxin producers and their growth under certain climatic conditions has led to fumonisin concentrations of up to 117 ppm in corn [3]. Of the more than 15 fumonisin isomers that have been described so far, fumonisins B_1 (FB_1), B_2 (FB_2), and B_3 (FB_3) are the

Correspondence can also be addressed to: Dr. Ken Voss, USDA-Agricultural Research Service, Russell Research Center, PO Box 5677, Athens, GA 30604, USA
E-mail: KVoss@saa.ars.usda.gov
Fax: +1-7065463116

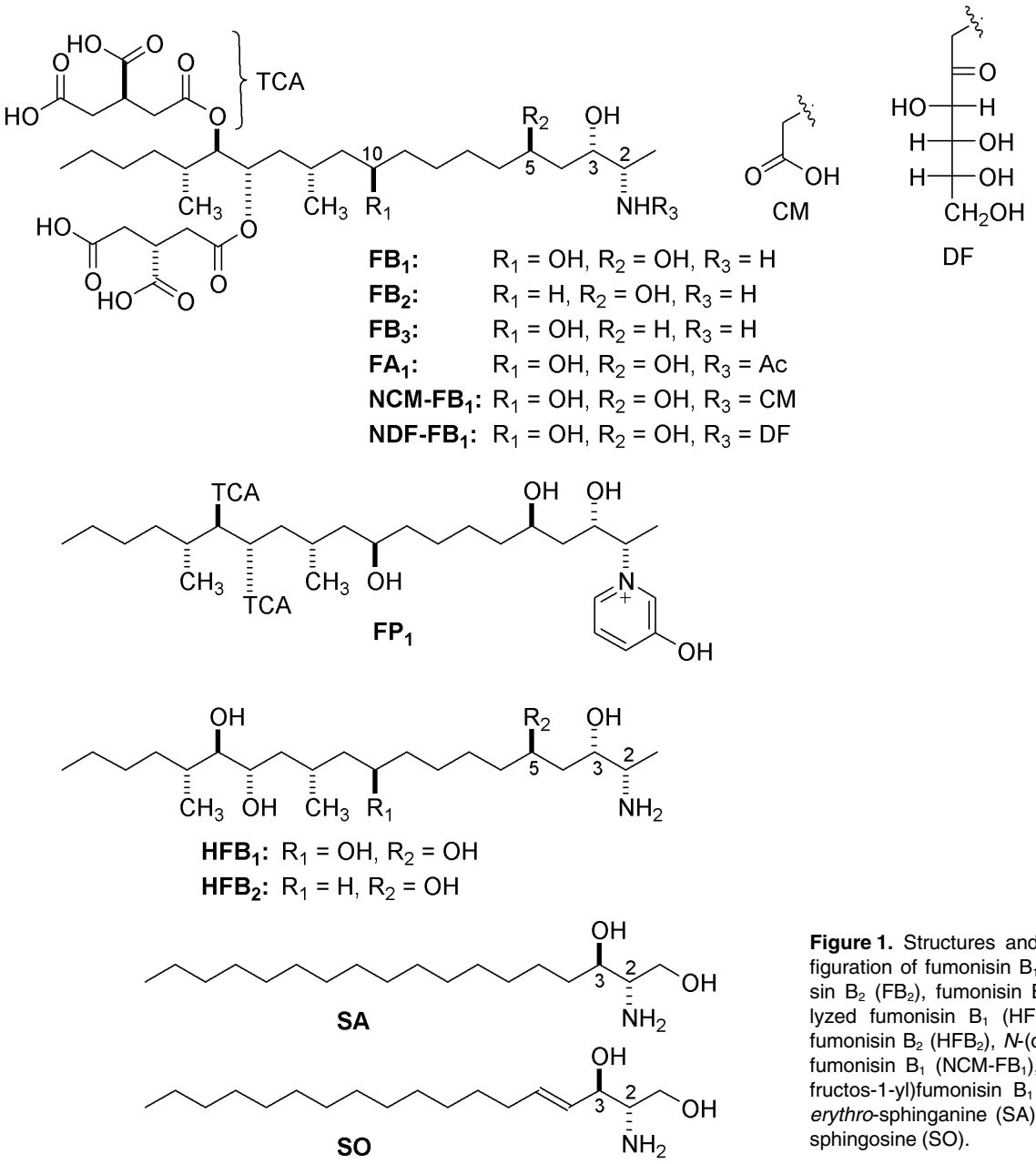

Figure 1. Structures and absolute configuration of fumonisin B_1 (FB_1), fumonisin B_2 (FB_2), fumonisin B_3 (FB_3) hydrolyzed fumonisin B_1 (HFB_1), hydrozyed fumonisin B_2 (HFB_2), *N*-(carboxymethyl)-fumonisin B_1 (NCM-FB_1), *N*-(1-deoxy-D-fructos-1-yl)fumonisin B_1 (NDF-FB_1), D-*erythro*-sphinganine (SA) and D-*erythro*-sphingosine (SO).

most abundant (Fig. 1). Fumonisins (FB_x) are diesters of propane-1,2,3-tricarboxylic acid (TCA) and similar long-chain aminopolyol backbones (for FB_1: 2*S*-amino-12*S*,16*R*-dimethyl-3*S*,5*R*,10*R*,14*S*,15*R*-pentahydroxyeicosane). Structurally, fumonisins resemble the sphingoid bases sphinganine (SA) and sphingosine (SO) to which TCA groups have been added at the C-14 and C-15 positions (Fig. 1). Fumonisin B_1 contains 10 stereocenters (1024 different possible stereoisomers); intensive studies by several research groups have determined the absolute configuration (Fig. 1) [4, 5]. Interestingly, the biological activity of fumonisins depends on their absolute configuration as shown in experiments comparing synthetic analogs having different stereochemistries [6]. Other structural features especially an unsubstituted primary amine group are also important for biological activity [7, 8].

Toxicological assessment of fumonisins has for the most part centered on fumonisin B_1, the most prevalent isomer in nature. Thorough reviews with extensive reference lists have been published [9, 10]. Data for other fumonisins are limited (Table 1). Fumonisin B_1 causes leukoencephaloma-

Table 1. Summary of reported toxicological effects of selected fumonisins and fumonisin reaction products

Fumonisin	Toxicity/biological activity	Comments	Reference(s)
FB_1	Equine leukoencephalomalacia; Porcine pulmonary edema; Liver and kidney apoptosis, regenerative lesions (multiple species); Liver and kidney carcinogen (rodents); Inhibits ceramide synthase-disrupts lipid metabolism and function; Inhibits folate uptake *in vitro* and *in vivo* in LMBc mice.	Most common analogue; Found in corn and cooked products; Water-soluble; Possible human carcinogen.	[1, 2, 10 – 14, 25, 26, 113, 114]
FB_2/FB_3	Equine leukoencephalomalacia (FB_2 in naturally contaminated feed); Liver and kidney toxicity and ceramide synthase inhibition as per FB_1 *in vivo* (rats fed culture materials containing FB_2 or FB_3 for 21 days); No toxicity found in mice (FB_2 or FB_3-28 day feeding studies); Inhibit ceramide synthase *in vitro*.	Co-occurs with FB_1 but usually at lower concentrations; Less well-studied but toxicity similar to FB_1.	[7, 42, 90, 91, 115]
Hydrolyzed FB_1 (HFB)	Liver tumor promotion in rats (hydrolyzed culture material feeding study); Liver and kidney toxicity, ceramide synthase inhibition in rats (hydrolyzed culture material feeding study); No toxicity found in rats or mice (short-term feeding studies); Inhibits ceramide synthase *in vitro* less potently than FB_1; More potent than FB_1 in some *in vitro* toxicity assays (LDH release); Substitutes for sphingoid base as substrate for ceramide synthase catalyzed formation of *N*-acyl aminopentols, *in vitro* and *in vivo* in rats.	Formed by alkaline hydrolysis of FB, hydrolysis removes TCA groups; Found in masa and other nixtamalized products; HFB_2, HFB_3, *etc.* also form, biological activity of HFB_2, *etc.* not studied.	[6, 7, 41, 83, 89, 90 – 95]
N-Acetyl FB_1 (FA_1)	Not toxic to mice (28-day feeding study); Does not inhibit ceramide synthase *in vitro*; In contrast to FB_1, FA_1 (and FA_2) had no liver cancer initiation/promotion activity in rats.	Occurs naturally in minor amounts; Rearranges to *O*-acetyl FB_1, which inhibits ceramide synthase *in vitro.*	[8, 90, 91]
Browning reaction products	Reduced toxicity relative to FB_1 when fed to rats (reaction products not identified, likely NDF- or NCM-FB_1)	Form when FB_1 is heated in the presence of glucose/other reducing sugars; Initial product is likely a Schiff base that undergoes rearrangement (see NDF-FB_1 and NCM-FB_1 below).	[100, 104, 105]
N-(1-Deoxy-D-fructose-1-yl) FB_1 (NDF-FB_1)	Unknown; likely to be nontoxic and to not inhibit ceramide synthase (*N*-substitution reduces activity).	Forms when FB_1 reacts with reducing sugar (Browning reaction); First stable fumonisin Browning reaction product to be identified; Forms through Schiff base, NDF-FB_1 then rearranges to NCM-FB_1; NDF-HFB_1 forms by alkaline hydrolysis of NDF-FB_1; Negligible amounts NDF-fumonisins found in foods.	[95, 96]
N-(Carboxymethyl) FB_1 (NCM-FB_1)	No effects in mice (28-day feeding study); Does not inhibit ceramide synthase.	Occurs naturally in minor amounts; Forms by rearrangement of NDF-FB_1; Analogous NCM-HFB_1 forms as result of base hydrolysis; Traces found in nixtamalized products.	[91, 95, 96, 99]
FB_1-starch or FB_1-protein conjugates	Unknown, but FB_1 is potentially bioavailable if conjugates break down in gastro-intestinal tract.	FB_1 binds to model starch or model proteins when heated; Presence of these compounds in foods not proven but compounds of this type are likely explanation for "hidden" fumonisins liberated as hydrolyzed forms from cornflakes by base hydrolysis.	[57, 60, 110, 111]
FP_1	No effects in mice (28-day feeding study).	Amine group of FB_1 converted by *Fusarium* spp. to 2-hydroxypyridine under anaerobic conditions.	[91, 116]

lacia in horses [11, 12] and pulmonary edema in swine [13, 14], two fatal diseases in animals long associated with the consumption of *F. verticilllioides*-contaminated corn. Fumonisin B_1 is hepatotoxic and nephrotoxic to a variety of other species [1, 10] and is a liver and kidney carcinogen in rodents [15 – 17]. However, while clearly a human health concern [2, 9], no unequivocal relationship between fumonisins and disease in man has been established. Nonetheless, surveys showing a positive correlation between dietary fumonisins and human esophageal cancer rates in areas of Africa and China have been reported [18, 19]. It has also been hypothesized that fumonisins are a risk factor for neural tube defects (NTDs, a type of birth defect) in some populations exposed to high dietary levels through the consumption of foods prepared from contaminated corn [20, 21]. Results of rat [22], mouse [23], and rabbit [24] reproductive studies were negative for teratogenicity. However, a model using inbred LMBc mice for the induction of NTD

Table 2. Effects of processing methods on the fumonisin content of food

Method	Effect	Ref.
Milling		
Dry milling	Fumonisins were found in the germ, bran, and fines of corn. Flaking grits contained low levels of fumonisins.	[61]
Wet milling	Starch did not contain detectable FB_x residues. Fumonisin levels in other fractions were in the order of gluten > fiber > germ.	[63]
Cleaning		
Sieving (3mm screen)	Sieving out 'fines' (<3mm) from intact corn kernels (0.53 – 1.89 mg total fumonisins/kg) reduced fumonisin levels (FB_{1-3}) by 26 – 69%.	[62]
Heating		
Boiling (100°C, 30 min)	Boiling *Fusarium verticillioides* culture material did not reduce FB_1 concentraton.	[64]
Pasteurization (62°C, 30 min)	Pasteurization at 62°C for 30 min had no effect on loss of FB_1 or FB_1 spiked into milk (50 ng/mL).	[66]
50 – 150°C (0 – 960 min)	The decrease of FB_1 in dry corn (1530 mg FB_1/kg) heated at 75 – 150°C followed first order kinetics.	[67, 68]
190°C (60 min) 220°C (25 min)	Heating corn meal and moist corn meal at 190°C resulted in 20 – 40% recovery of FB_1 and FB_2. At 220°C recovery was 0%.	[69]
100 – 235°C; 0 – 60 min; pH 4,7 and 10	FB_1 and FB_2 were least stable in aqueous solution at pH 4 followed by pH 10 and 7; Decomposition began at temperatures ≥150°C. At T ≥175°C over 90% of FB_x were lost after 60 min regardless of pH.	[70, 71]
74 – 78°C, 14 – 18 h, pH 7.5	Incubation of FB_1 and D-glucose in aqueous solution resulted in the formation of NCM-FB_1.	[99]
100°C, 20 min	Using a traditional recipe for the preparation of South African maize porridge a mean reduction in FB_1 levels of 23% was observed.	[65]
79°C, 17h, pH 7.5	Incubation of FB_1 and D-glucose in aqueous solution resulted in the formation of NCM-FB_1 and NDF-FB_1.	[96]
Dry heating: 105 – 160°C, 3 – 40 min	Model experiments using methyl-α-D-glucopyranoside (starch model) and protected amino acids (protein model) show the covalent binding of FB_1 to polysaccharides (5 – 10%) and proteins *via* the TCA side chains.	[60]
Extruding		
120 – 160°C, 18 – 26% moisture level	Loss of FB_1 using mixing screws: 29 – 69% (ELISA) and 31 – 68% (HPLC), using nonmixing screws: 13 – 54% (ELISA) and 20 – 47% (HPLC).	[75]
140 – 200°C	Extruding of corn grits spiked with 5 mg FB_1/kg resulted in 34 – 95% loss of FB_1.	[76]
150 – 180°C, 14% moisture level	70 – 90% loss of FB_1 and FB_2 when corn flour was extruded in a single screw extruder.	[77]
70 – 105°C, 5 min, ~27% moist. lev., then roasting: 170–220°C, 50s	About 60 – 70% of the initial amount of FB_1 and FB_2 were lost during the entire cycle (extruding and roasting) of corn flakes processing.	[79]
Extrusion (160 – 180°C, 16 – 20% moisture) and gelatinization (90 – 110°C, 24 – 30% moisture) of spiked corn grits (2 mg/kg FB_1, 0.6 mg/kg FB_2) reduced fumonisin levels by ~45 – 70%, cooking (130°C, 30 – 90 min) the grits for flaking ~35 – 80% and roasting (250°C, 2.5 – 5 min) ~65 – 94%.		[80]
140 – 180°C, 26% moisture level, screw speed: 40 – 120 rpm, 0 – 10% sugar	Extrusion cooking of corn grits reduced FB_1 depending on the added sugar (glucose: 45 – 71% reduction of FB_1, fructose: 30 – 53%, sucrose 19 – 39%). Baking corn muffins (200°C, 30 min) with added glucose also reduced FB_1 levels by up to 50%.	[56]

Table 2. Continued

Method	Effect	Ref.
Extruding (continued)		
160–180°C, 16–20% moisture level, screw speed: 180–220 rpm, 5% sugar	Extrusion processing of spiked corn grits (2 mg/kg FB_1) led to the formation of NCM-FB_1. The total recovery of FB_1 (FB_1 and NCM-FB_1) was 23–32% in sucrose and 7–15% in glucose spiked samples. Therefore 68–93% of FB_1 were lost during processing.	[81]
171°C, 24–33% moisture level, screw speed: 122 rpm	Extrusion processing of alkali-cooked corn meal reduced FB_1 (2–99% reduction) and HFB_1 (8–67% reduction) levels. The use of a tapered-angular die reduced FB_1 and HFB_1 levels more than a tapered-circular die.	[78]
Baking (B), Frying (F) and Roasting (R)		
B: 175 and 200°C (20 min) F: 140–190°C (0–15 min)	Baking corn muffin mix spiked with 5 mg/kg FB_1 resulted in 16–28% loss of FB_1. FB_1 began to degrade when corn chips were fried at temperatures $\geq$ 180°C and times $\geq$ 8 min.	[58]
B: 204 and 232°C (20 min) R: dry heat, 218°C, 15 min	Baking corn muffin mix (spiked with 5 mg FB_1/kg) lead to no significant loss of FB_1 at 204°C; 48% loss of FB_1 at 232 °C. Roasting corn meal (5 mg FB_1/kg) resulted in a complete loss of fumonisins.	[72]
B: 210°C, 25 min	Baking of artificially (15 µg ^{14}C-FB_1 or FB_1/muffin) and naturally contaminated (1.56 µg ^{14}C-FB_1/muffin) corn muffin mixes resulted in significant losses of FB_1 (57, 52, and 51%) as measured by HPLC.	[73]
B: 190°C,1 h F: 218°C, 10–12 min (panfry); 193°C, ~10 min (deep fry)	Baking and frying of cornmeal (contaminated with high levels of fumonisins) had no significant effect on *in vivo* toxicity to rats. Study results provided no evidence that fumonisins were converted to novel toxins.	[98]
Nixtamalization (NM)		
NM: 100°C, 5 min; frying: 190°C, 60 s	Nixtamalization of contaminated corn (8.79 mg/kg FB_1) followed by the production of tortillas reduced the initial FB_1 concentration by 81.5%. (H)FB_1 was mainly found in the steeping and washing water.	[93]
NM: 100°C, 105 min; cooking: 170–212°C, ~3.5 min	Using the traditional nixtamalization method of Mayan communities the total fumonisins (HFB_1 + FB_1) in tortillas were reduced by 50%. The residual lime and washing water also contained 50% of the total FB_1 in the starting material (38.1 mg/kg).	[94]
NM: commercial conditions	Production of fried tortilla chips in a pilot production line reduced FB_1 up to 80% compared to the raw corn. Chips contained HFB_1 in low concentration (ppm) and almost no NCM-FB_1 and NDF-FB_1. Cooking and steeping the corn in water was the critical step for reducing fumonisins in the masa and chips.	[95]

by fumonisin B_1 *in vivo* has recently been developed [25] and results of these experiments have provided a plausible mechanism for NTD development in which decreased folate utilization (folate deficiency is a risk factor for NTD) is mediated by fumonisin-induced disruption of sphingolipid metabolism (see below) [21].

Fumonisins disrupt *de novo* sphingolipid biosynthesis and metabolism [26–28]. Because of their structural relationship to the sphingoid bases SA and SO, fumonisins competitively inhibit ceramide synthase, a key enzyme in sphingolipid metabolism, which catalyzes the acylation of SA, SO, and other sphingoid bases [26–29]. As a consequence, cellular SA levels increase dramatically, as does the SA/SO ratio. Both SA and the SA/SO ratio are used as a biomarker of exposure and elevated SA and increased SA/SO ratios have been consistently observed in various animal species [30–42] as well as different cell lines experimentally exposed to fumonisins [7, 8, 45–47]. The usefulness of SA or SA/SO as a biomarker for animal exposures in the field or in humans has not yet been validated [43, 44]. The inhibition of ceramide synthase disrupts sphingolipid metabolism, causing an elevation of SA, which is a highly bioactive compound, a reduction in complex sphingolipids and otherwise initiating cellular events that are thought to be ultimately responsible for the toxicity and carcinogenicity of this mycotoxin [6, 27–29].

On the tissue level, apoptosis is usually the initial finding in liver, kidney, and other affected organs [10]. However, with

time or increasing dose both cell death (apoptosis and necrosis) and replication/compensatory regeneration typically occur together in liver and kidney [10, 48, 49]. It has been hypothesized that consistent compensatory regeneration in response to cell loss over time is critical for carcinogenesis [48, 50]. However, while it is clear that SA or SA/SO increases occur in tissues at doses at or below the lowest observed affect level for apoptosis and that the severity of tissue injury and increased SA/SO are correlated *in vivo* [28, 36, 38–42], the sequence of events linking ceramide synthase inhibition to apoptosis and mitosis/regeneration in liver or kidney remains to be elucidated. TNF-α signaling and caspase activation is involved [38–40, 51], however, recent data suggest that TNF-α signaling modifies, but is not obligatory for fumonisin-induced apoptosis in mouse liver [40, 52]. Changes in gene expression for proteins involved in the regulation of cell cycle progression such as cyclin D1 [53, 54], cyclin E, p21, p27 [54], c-myc, and hepatocyte growth factor (or stabilization of these proteins) have also been found to occur in response to fumonisin exposure [49].

Approximately 600 million tons of corn (called maize in much of the world) are produced each year worldwide and most of it (*ca.* 63%) is directly used for animal feed [55]. Only approximately 25% is used for food, of which the majority is manufactured into food products or ingredients using physical or chemical processing methods (see Table 2). Relatively few studies on how cooking affects fumonisins have been reported. It has been shown that cooking reduces the concentration of fumonisins in food products and that in some methods, particularly extrusion or nixtamalization, reductions might be significant from a toxicological standpoint. However, in most cases, including extrusion, it has not been established whether fumonisin reductions result from their thermal decomposition or from binding of the mycotoxin to food matrix components [56–58]. This is an important consideration as illustrated by the results of McKenzie *et al.* [59], who showed that a novel reaction product resulting from ozone treatment (a proposed fumonisin detoxification method) of fumonisin B_1 retained the mycotoxin's biological activity. More recently, evidence for the presence of matrix-bound or "hidden" (not extracted with routinely used solvent systems) fumonisins in foods is accumulating [57, 60]. The toxicological implications of any "hidden" fumonisins would depend not only on their concentration in foods, but also on the extent to which they are made bioavailable during digestion. Concerning food safety, it is therefore important to know what happens to fumonisins during food manufacturing. In this review, the results of experiments to determine how cooking and processing affect fumonisins will be considered and the toxicological implications of these findings will be discussed.

2 Effects of processing

2.1 Milling and cleaning

The basic raw materials for breakfast cereals, snacks, and many other corn products are cornmeal, flour and grits. These ingredients are produced by dry milling of corn kernels. Katta *et al.* [61] studied the fate of fumonisin B_1 in naturally contaminated corn samples during the dry milling process. It was shown that the bran fraction, which is used as animal feed, contained the highest concentration of fumonisins, followed by the germ, whereas the cornmeal, flour, and grit fractions used for food production contained little or no fumonisin. Furthermore, it was shown that cleaning to remove broken kernels and other material < 3 mm in size, which is usually the first step in the processing of corn, reduces fumonisin levels from 26 to 69% [62]. Another basic, widely used ingredient for food is corn starch, which is produced by wet milling. During this process fumonisins are dissolved into the steepwater or distributed to the gluten, fiber, and germ byproducts which are used as animal feed, leaving no detectable amounts in the starch. Fumonisin concentrations in gluten, fiber, and germ ranged from about 10% (germ) to 40% (gluten) of those in the raw corn kernels [63].

2.2 Thermal treatment

Fumonisins are relatively heat-stable (up to 100–120°C) and therefore survive many of the conditions used in cooking and food manufacturing. In one of the first fumonisin stability studies, Alberts *et al.* [64] found that boiling *Fusarium verticillioides* culture material for 30 min did not reduce its FB_1 concentration. However, in a more recent study, a moderate reduction in fumonisin levels was achieved under similar conditions; specifically, the preparation of South African maize porridge using a traditional recipe (boiling salted corn meal for 20 min in water) resulted in a 23% reduction in its concentration [65]. Pasteurization of milk spiked with 50 ng/mL FB_1 and FB_2 at moderate temperatures (62°C, 30 min) did not significantly reduce fumonisin levels [66]. Decomposition of fumonisins begins at higher temperatures (see Table 2) and thermal decomposition of FB_1 in dried corn culture material followed a first order reaction with half-life times of 175 min at 100°C, 30 or 38 min at 125°C, and 10 min at 150°C [67, 68]. Heating dry corn meal and moist corn meal at 190°C for 60 min resulted in 60–80% loss of fumonisins while after baking at 220°C (25 min), loss of FB_1 and FB_2 was almost complete [69]. The thermal instability of fumonisins was further demonstrated in a model experiment in which they were heated in the absence of corn matrix. Specifically, when the stability of FB_1 and FB_2 in aqueous buffered solutions at pH 4, 7, and 10 was determined as a function of the

processing time (0–60 min) and temperature (100–235°C), FB_1 and FB_2 were least stable at pH 4 followed by 7 and 10. Decomposition began at or above 150°C and, at temperatures ≥175°C, over 90% of the fumonisins were lost after 60 min regardless of pH [70, 71].

The effects of baking, frying, and roasting on the stability of fumonisins have also been examined by several research groups. Baking corn muffin mix spiked with 5 mg/kg FB_1 at 175 or 200°C resulted in a 16–28% loss of FB_1, however, the effect was not uniform and loss was significantly higher at the surface than in the core of the muffin [58]. In another study, baking a corn muffin mix (5 mg/kg FB_1) at 204°C did not significantly reduce FB_1, whereas when baked at 232°C, 48% of the FB_1 was lost [72]. Frying masa at 140–170°C for 0–6 min resulted in no significant loss of FB_1 but the mycotoxin began to degrade at frying temperatures at or above 180°C and cooking times of 8 min or longer [58]. Roasting corn meal spiked with 5 mg/kg FB_1 at 218°C for 15 min resulted in a complete loss of fumonisins [72]. Baking muffins using a cornmeal muffin mix spiked with 15 μg ^{14}C-FB_1/muffin or 15 μg unlabeled FB_1/muffin resulted in significant losses of FB_1 (52–57%) as measured by HPLC [73]. When muffins were baked using contaminated cornmeal (1.56 μg ^{14}C-FB_1/muffin) prepared from kernels colonized by *F. verticillioides* (^{14}C-FB_1 was synthesized by the fungi from 1,2-^{14}C-sodium acetate), a similar result (51% loss) was obtained by HPLC analysis. However, when the amount of radioactivity (assumed to be labeled FB_1 or labeled FB_1-like compounds) in the muffin extracts was determined, considerably more label (90%) was recovered from muffins baked with the cornmeal prepared from the fungus-colonized kernels than from muffins made from spiked cornmeal (52% recovery). These results are important because they demonstrated the existence of a fumonisin-like compound(s) in the muffins made from the colonized kernels that could not be quantified using a standard extraction/immunoaffinity column cleanup/HPLC procedure [73].

One of the most important technologies for the food industry is extrusion processing which has been used since the mid 1930s for the production of breakfast cereals, snack foods, and textured foods [74]. Other applications of extrusion processing include dry and soft moist pet food, precooked and modified starches, flat bread, precooked noodles, soups, and other products. During extrusion cooking, the raw material is subjected to high temperature, high pressure, and severe shear forces. These variables as well as the moisture level of the raw material are important for determining the physical properties of the product. Two major types of extruders are used in the food industry: single-screw and twin-screw extruders. In an extruder, a semisolid homogeneous mass of the raw materials is formed under a variety of controlled conditions and then pressed by a rotating screw through a restricted opening (die) such as a shaped hole or slot into a pressure free space. Mechanical energy is converted to heat and additional heat can also be applied in the form of steam to the extruder barrel (fits tightly around the rotating screw). As a result, the temperature in the extruder can be as high as 200°C even though the residence time of the dough in the machine is relatively short (10–60 s). In addition, the high pressure and severe shear forces that are generated contribute to chemical reactions and molecular modifications that occur in the dough, *e.g.*, starches are gelatinized, proteins are denaturated, and enzymes are inactivated. Furthermore, the products expand due to the high-speed evaporation of water, which happens as the products emerge from the extruder [74]. Several studies have shown that extrusion processing significantly reduces measureable fumonisin residues in food products.

The first study by Castelo *et al.* [75] showed that the loss of FB_1 from corn grits at extruding temperatures between 140 and 160°C was significantly higher ($p < 0.05$) when using an extruder equipped with a mixing screw (29–69% loss) than one fitted with a nonmixing screw (13–54% loss). A linear decrease in FB_1 levels was observed with nonmixing screws and as the moisture content of the corn grits increased. However, the amount of reduction found also depended not only on the cooking conditions but also on the analytical procedure, *i.e.*, the extraction solvent and quantification method used. Another study examined the effect of barrel temperature (140, 160, 180, and 200°C) and screw speed (40, 80, 120, and 160 rpm) on the stability of FB_1. The corn grits were spiked with 5 mg FB_1/kg and cooked in a co-rotating twin-screw extruder [76]. Both parameters affected the fumonisin reductions: FB_1 recovery from the extruded corn grits decreased as the temperature increased and as the screw speed decreased. Depending on the conditions, the reductions of FB_1 ranged from 34 to 95% and, when the grits were processed under conditions that resulted in an acceptable product, reductions were in the range of 46–76% [76]. Similarly, Pineiro *et al.* [77] found 70–90% loss of FB_1 and FB_2 when corn flour was extruded using a single screw extruder at 150–180°C. Cortez-Rocha *et al.* [78] studied the influence of die geometry on recoverability of FB_1 and HFB_1 in alkali-cooked corn flour. Extrusion processing using a twin-screw extruder (171°C, screw speed 122 rpm) fitted with a tapered-angular die (3 mm opening) or a tapered-circular die (5 mm opening) reduced FB_1 from 2–99% and HFB_1 from 8–67%. Reductions tendered to be higher when a tapered-angular die was used [78].

The effect of processing on fumonisin levels in corn flakes was studied by De Girolamo *et al.* [79], who reported that 60–70% of the initial amount of FB_1 and FB_2 was lost during the entire corn flakes processing cycle (extruding and

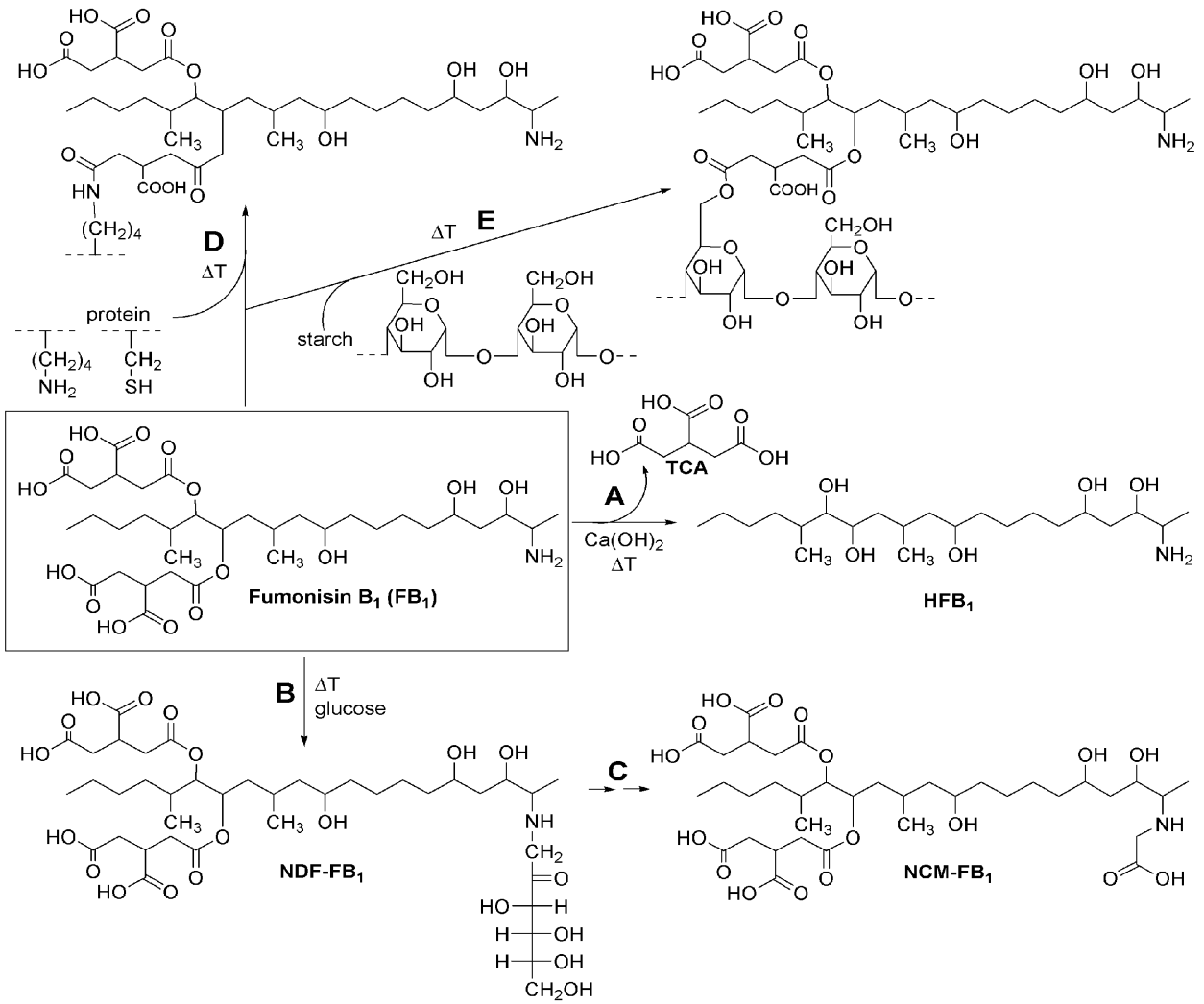

Figure 2. Structures of fumonisin B_1 degradation products and matrix-bound fumonisin B_1 formed under thermal processing conditions (for details see text).

roasting). Loss of fumonisins during the intermediate extrusion step of the cycle (70–105°C, 5 min, ~27% moisture level) was less than 30%. Loss of fumonisins was also observed by Meister [80]. Extrusion cooking (160–180°C, 16–20% moisture level) and gelatinization (90–110°C, 24–30% moisture level) of spiked corn grits (2 mg/kg FB_1, 0.6 mg/kg FB_2) reduced fumonisin levels by ~45–70%, cooking (130°C, 30–90 min) the grits for flaking by ~35–80%, and roasting (250°C, 2.5–5 min) by ~65–94% depending on the selected technological parameters [80].

Seefelder *et al.* [81] and Castelo *et al.* [56] studied the effects of added sugars on fumonisin levels in extruded corn grits. Both found significant reductions of fumonisin concentration in the extruded product, however, the amount of reduction depended on the type and amount of sugar added. Extruding corn grits that were spiked with 2 mg/kg FB_1 resulted in a 68–77% reduction of FB_1 with added sucrose and in a 85–93% reduction of FB_1 with added glucose (parameters see Table 2) [81]. Castelo *et al.* [56] found a fumonisin reduction of 45–71% when glucose, 30–53% when fructose and 19–39% when sucrose was added. In a second experiment, the effect of various screw speeds (40–80 rpm) and glucose concentrations (2.5–10%) on fumonisins in corn grits extruded at 160°C were evaluated. As expected, both parameters affected FB_1 with slow screw speed and higher glucose concentration increasing reduction [56].

Another important process is alkali cooking or nixtamalization. It is used to produce snacks and tortilla products and consists of first cooking corn in alkaline water for a short period of time and then steeping it overnight (for more details see the review by Saunders *et al.* [82]). Under alkaline conditions, fumonisins in contaminated corn are converted to the so-called hydrolyzed fumonisins (HFB_x) (Fig. 1), an amino-

pentol moiety (AP_x) formed by hydrolytically removing the two tricarballylic acid side chains from the 20-carbon fumonisin backbone (Fig. 2, reaction A) [83 – 86].

Hydrolyzed fumonisin B_1 (HFB_1) occurs in nixtamalized corn products (such as masa and tortilla chips) and canned yellow corn, but usually at lower concentrations than FB_1 [84, 87, 88]. The toxicological significance of hydrolyzed fumonisins in foods is not yet clear. HFB_1 inhibited ceramide synthase *in vitro* less effectively than FB_1 [7], suggesting that HFB_1 was potentially less toxic *in vivo*. When fed to rats, nixtamalized corn culture materials (of fumonisin-producing *Fusarium* spp.) that contained HFB_1 and HFB_2 but no detectable FB_1 or FB_2 [41, 83, 89] did elicit hepato- and nephrotoxic effects that were qualitatively similar to those found in rats fed with equal amounts of the untreated culture material. However, as predicted by the *in vitro* results, target organ toxicity, as judged by histopathological criteria and increased tissue concentrations of sphingoid bases, was less severe in the rats fed nixtamalized culture material than in those fed the untreated material containing FB_1 and FB_2 [42, 89]. The concentration of HFB_1 in the diets containing the nixtamalized culture material (140 μM/kg diet) was higher than the FB_1 concentration of the diets prepared with untreated culture material (98 μM/kg diet). This finding, in conjunction with the toxicology results, is further evidence that HFB_1 is a less potent toxin than FB_1 *in vivo*. HFB_1 also failed to initiate cancer in rats (FB_1 had weak initiating activity) using a short-term liver cancer initiation/promotion model [90]. Howard *et al.* [91] fed diets containing equivalent amounts (0, 14, 70, and 140 μM/kg diet) of purified FB_1 or HFB_1 to female mice for four weeks. Liver histopathological and sphingoid base effects were found only in mice fed the mid- and high-dose FB_1 diets. HFB_1 had no effect on these variables.

Conversely, while the weight of evidence suggests that hydrolyzed fumonisins are less toxic *in vivo* than their corresponding fumonisin analogues, HFB_1 has been shown to have greater cytotoxicity than FB_1 *in vitro* (primary hepatocyte cultures) [90]. Furthermore, *in vitro* experiments showed that HFB_1 and HFB_2 not only inhibit ceramide synthase, but are also substrates that are acylated with several fatty acyl-CoA's by the enzyme to their corresponding *N*-acyl HFB_x derivatives, which were more toxic to HT29 cells in culture compared to HFB_1 [6]. The formation of *N*-acyl HFB_1 has been detected *in vivo* (rat liver) but the potential significance of such metabolites for toxicity is still unclear [92]. The combined effect of nixtamalization and cooking on fumonisin levels has been investigated by several research groups and, in each case, the concentration of fumonisins in the product was less than in the uncooked corn. Dombrink-Kurtzman *et al.* [93] showed that nixtamalization reduced the FB_1 concentration in tortillas by 81.5% and that the FB_1 and HFB_1 were mainly found in the steeping and washing water [93]. Cortez-Rocha *et al.* [78] observed a 39% reduction of FB_1 concentration when raw corn was nixtamalized (1,2% $Ca(OH)_2$, 55 min, 95–100°C). Others found that the traditional nixtamalization method (*ca.* 400 g corn, 0.2 L lime solution (82 g CaO/L), 1.1 L water, 100°C, 105 min) used by the Mayan communities in Guatamala reduced total fumonisins (FB_1 and HFB_1) by 50% [94] and that the residual lime and washing water also contained 50% of the fumonisins initially present in the corn.

Differences exist between nixtamalization as practiced in the home or other small-scale situation and as done in a large-scale industrial setting [82]. During the commercial production of fried tortilla chips, the corn is first nixtamalized by cooking/steeping in alkaline water. The nixtamalized corn is rinsed and ground into masa and the masa is then sheeted, cut, baked, and fried. As a result, the concentration of fumonisins in the fried chip product was considerably reduced relative to that in the raw corn [95]. The amount of reduction varied in four batches of chips, ranging from 50 to 78% for the three batches of chips prepared from corn containing >1 ppm fumonisin B_1 and 37% for the batch prepared from corn which had low (<0.25 ppm; fumonisin B_1) content. As in the other investigations cited above, reduction was achieved as a result of fumonisins being diverted from the product stream by extraction into the cooking/steeping liquid. The extracted fumonisins were mostly (about 88% at the end of the cooking/steeping step) hydrolyzed while, in contrast, most fumonisins remaining in the masa (63%) were unchanged. Baking and frying the masa had no meaningful effect on fumonisin concentrations and no significant amounts of the fumonisin-reducing sugar browning reaction products *N*-(carboxymethyl)- or *N*-(deoxy-D-fructos-1-yl)-fumonisins were found. A mass balance estimate was done for one of the batches. About 80% of the fumonisins originally present in the raw corn was found: approximately 35% in the masa and *ca.* 45% in the cooking/steeping liquid. The identity and fate of the remaining 20% was not determined. The *in vitro* biological activity (ceramide synthase inhibition) and fumonisin B_1 concentration (HPLC) of the corn, masa, and baked and fried chips were correlated [97, 98]. However, both the bioassay and standard HPLC quantification methods for fumonisins depends upon their extractability from the corn or food matrix and, therefore, the bioavailability or toxicity of any fumonisins which remained tightly bound to the masa or tortilla chip matrices could not be predicted on the basis of this *in vitro* bioassay.

Most of the data indicate that fumonisin levels are decreased during heating, baking, frying, roasting, nixtamalizing, and extrusion cooking of foods. The amount of reduction is, however, variable and influenced by cooking conditions, such as temperature, time, water and sugar content, and pH. In most

of the aforementioned studies, the fate of the fumonisins was not determined. Theoretically, loss of fumonisins during processing may indicate that they are (i) extracted or otherwise removed from products, (ii) destroyed, (iii) modified to form novel fumonisins, (iv) bound to matrix components or (v) otherwise rendered less extractable. Therefore, it cannot be assumed that reduced fumonisin levels are indicative of reduced toxicity unless the removal of fumonisins from the food during cooking has been unequivocally demonstrated. To understand the implications of the observed reductions in fumonisin concentrations which occur during cooking, it is necessary to clarify both the chemical structures and biological activities of fumonisin degradation products or matrix-bound fumonisins formed during thermal processing. Fumonisin reaction products which have been identified are summarized in Fig. 2.

3 Fumonisin reaction products and matrix binding

The first fumonisin degradation products described in the literature were the hydrolyzed fumonisins (HFB_x, Fig. 1). Hydrolyzed fumonisins have already been discussed in detail above and are an excellent example illustrating how reductions in fumonisin concentration and toxicity can be achieved by a combination of extraction and conversion of fumonisins to less biologically active forms. Another possibility is that, during food processing, fumonisins bind to matrix components or react with other ingredients. One such binding product is *N*-(carboxymethyl)fumonisin B_1 (NCM-FB_1) (Fig. 1), which is a stable reaction product formed when FB_1 is heated in the presence of reducing sugars [99]. The structure of NCM-FB_1 was identified by Howard *et al.* [99]. It has been shown in model experiments that, under moderate conditions, heating (60 – 80°C) 20 mM (or 100 mM) glucose (or other reducing sugars) and 0.25 mM (or 1.39 mM) FB_1 in potassium phosphate buffer (pH 7 – 7.5) results in the formation of NCM-FB_1 [99, 100]. It was suggested that the reaction proceeds, as a stable Schiff base is created, through a common Maillard reaction (for reviews see [101, 102]) between FB_1 (an aliphatic primary amine) and a reducing sugar [103]. The initial reaction product of FB_1 and D-glucose has been isolated and identified as *N*-(1-deoxy-D-fructos-1-yl) fumonisin B_1 (NDF-FB_1, see Fig. 1) [60, 96]. The primary amino group of FB_1 (or HFB_1) reacts with the carbonyl group of D-glucose to yield a Schiff base which then undergoes Amadori rearrangement to NDF-FB_1 (Fig. 2, reaction B). Following the general Maillard reaction scheme [101, 102], it is assumed that NDF-FB_1 is further converted to NCM-FB_1 (Fig. 2, reaction C) [99, 100]. Intermediates formed during the rearrangement of NDF-FB_1 to NCM-FB_1 have recently been identified [100]. It was also shown that heating D-glucose with HFB_1 resulted in the formation of the corresponding product NDF-HFB_1 [60]. *N*-(Deoxy-D-fructos-1-yl)-HFB_1 has been detected in the cooking/steeping liquid used during nixtamalization and, in this case, the evidence suggests that it arises from the base hydrolysis of NDF-FB_1. While, from a structure-function standpoint, this reaction sequence is intriguing toxicologically (see below), little to no NCM-FB1 or NDF-FB_1 has been found in corn [95, 99] or nixtamalization products [95]. At higher temperatures, *e.g.*, under extruding conditions (see Table 2), only low concentrations (27 – 97 μg/kg) [81] of NCM-FB_1 were detected in a product made from cornmeal spiked with 2 mg/kg FB_1.

The toxicological data on *N*-substituted fumonisins is somewhat limited but indicates that their biological activity is reduced relative to their respective *N*-unsubstituted analogues. Using the *N*-acetyl derivatives of FB_1 and FB_2, it was shown that blocking the amino group prevents toxicity in primary rat hepatocyte cultures (LDH release) as well as *in vivo* (induction of GGT positive foci) [90]. Purified *N*-acetyl FB_1 is also not an inhibitor of ceramide synthase *in vitro* [7]. While *N*-acetyl FB_1 does not inhibit ceramide synthase in vitro [7], it can undergo spontaneous rearrangement to *O*-acetyl FB_1 under some conditions [8] and the ceramide synthase inhibitory activity of the latter analogue was indirectly demonstrated. Specifically, a mixture of *N*-acetyl and *O*-acetyl FB_1 inhibited ceramide synthase of rat liver slices *in vitro* but, after cleanup to remove the *O*-acetyl FB_1 from the mixture, ceramide synthase was not inhibited by the remaining *N*-acetyl FB_1 [8].

Modifying FB_1 by heating it in the presence of fructose or glucose did reduce its hepatotoxicity and liver cancer promotion activity in rats, although the chemical structures of the reaction products were not identified [104, 105]. Interestingly, biological activity was reduced even though the FB_1-fructose products are apparently absorbed to a greater extent than FB_1 by the rats [106, 107]. Using the brine shrimp assay for toxicity assessment, NCM-FB_1 was 100-fold less potent than FB_1 [108]. Howard *et al.* [91] fed a series of fumonisin analogues to female mice for 28-days and compared their toxicity using fumonisin-specific endpoints for hepatotoxicity. The *N*-substituted analogues, including *N*-acetyl FB_1, NCM-FB_1, and FP_1 (a fumonisin in which the C2 amine of FB_1 is replaced by 2-hydroxypyridine [91 (see Fig. 1), 116]) elicited no toxic response and did not inhibit ceramide synthase at levels ≤ 140 μM/kg diet, whereas the LOAEL (lowest observed adverse effect level) for FB_1 was 70 μM/kg. Together, results of these *in vitro* and *in vivo* investigations showed that *N*-substitution reduces the biological activity of fumonisins. Further evidence for the importance of fumonisins' amino group is the finding that deaminated FB_1 (produced by sodium nitrite treatment of mycotoxin) was, in contrast to FB_1, not toxic to *Hydra attenuate* [109]. *N*-Acyl HFB_1 is a possible exception as it has been found to be more toxic than HFB_1 to HT29 cells *in vitro* [6,

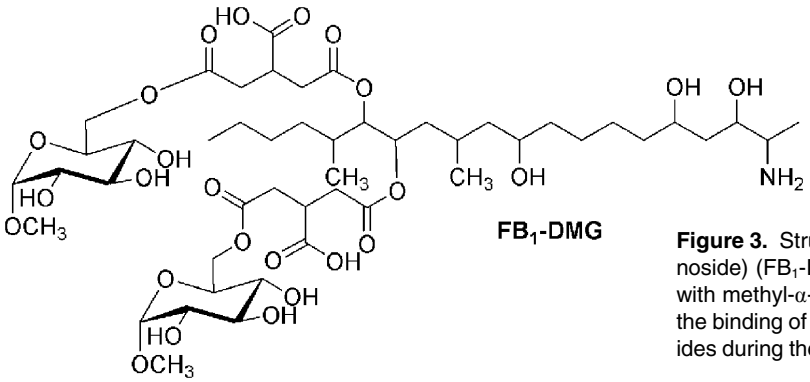

Figure 3. Structure of fumonisin B_1-di(methyl-α-D-glucopyranoside) (FB_1-DMG) resulting from the heating of fumonisin B_1 with methyl-α-D-glucopyranoside. The molecule demonstrates the binding of fumonisin B_1 *via* the TCA side chains to saccharides during thermal treatment [60].

29]. Additional studies are clearly needed to determine the toxicological significance of *N*-acyl HFB_1 and related compounds *in vivo*.

Besides the reactive amine group, fumonisins possess two TCA side chains (Fig. 1) as functional groups which might react with food matrix components. Shier *at al.* [110 – 112] partially characterized the covalent binding of radiolabeled fumonisin B_1 to corn proteins and starch. They proposed that binding resulted from a two-step reaction in which an anhydride is first formed by loss of a water from the TCA side chain, followed by a reaction between this anhydride and the free functional groups of starch (hydroxyl-groups) and proteins (amine or thiol groups) (Fig. 2, reaction D and E). To obtain more information about the binding of fumonisins *via* their TCA side chains to matrix components after thermal treatment (cooking), experiments using methyl-α-D-glucopyranoside as a starch model and amino acid derivatives (*N*-α-acetyl-L-lysine-methyl ester, BOC-L-cysteine-methyl ester) as protein models were performed recently [60]. As in starch, the anomeric carbon atom of methyl-α-D-glucopyranoside is protected and only the molecule's other hydroxy groups are available to react with FB_1. Likewise, only the thiol- or the ε-amino groups of the protected amino acids – similar to proteins – are free to react with fumonisins. The model compounds were heated (105 – 160°C, 3 – 40 min) with FB_1 and HFB_1 and the products which were formed under these conditions characterized by HPLC-ESI-MS/MS. Whereas a 5 – 10% yield of conjugated products was found following the reaction of FB_1 with methyl-α-D-glucopyranoside (starch model) or the amino acid derivatives (protein model), heating the model compounds in the presence of HFB_1 yielded no conjugation products, a finding which clearly indicated that binding of FB_1 to the model matrix components occurred *via* the TCA side chains. In these experiments, the highest yield (~10%) of conjugated product was obtained by heating methyl-α-D-glucopyranoside with FB_1. This reaction product was subsequently purified and identified by nuclear magnetic resonance (NMR) spectroscopy as fumonisin B_1-di-(methyl-α-D-glucopyranoside) FB_1-DMG (Fig. 3). While these studies using methyl-α-D-glucopyranoside and amino acid derivatives as model compounds have demonstrated that fumonisins are able to bind to polysaccharides and proteins *via* their two tricarballylic acid side chains [60], the extent to which these compounds occur in food products is not known.

It is expected that base hydrolysis of food products would release any starch or protein-bound fumonisins (*via* their TCA groups) as hydrolyzed analogues [60, 81, 110 – 112]. Kim *et al.* [57] applied this principle during a survey of cornflakes and found that secondary base hydrolysis of the extracted (initially extracted for fumonisin analysis) cornflakes liberated an additional, significant amount of fumonisins (2.6 times the originally measured amount) from the matrix, which they designated "hidden fumonisins". Because the cornflakes used in these surveys were purchased off the shelf, it is not known if the "hidden fumonisins" were formed by binding of fumonisins to the matrix during cooking/processing or were present in the raw corn prior to processing. While the exact nature of the bonds forming between fumonisins (*via* the TCA groups) and starches, proteins or other food matrix components remains to be elucidated, the presence of matrix-bound fumonisins is a potential food safety concern because of the possibility that free fumonisins, or their hydrolyzed analogues, could be released in the gastrointestinal tract [60, 110, 111]. If this is the case, then analysis of foods using standard protocols involving extraction/column cleanup/derivatization with a fluorophore/HPLC (or HPLC-MS) quantification might underestimate fumonisin content (and exposure to consumers) of some food products.

4 Conclusions

Since their discovery, a number of experiments on how milling, cooking, and other processing steps affect fumonisins in corn and corn-based products have been reported. Processing clearly affects fumonisins and the fumonisin concentration in a given feed or food product will be the net result of the affects of all the steps used in its preparation including: (i) physical removal of fumonisin-contaminated kernels (cleaning the corn to remove broken and moldy ker-

nels); (ii) differential distribution of fumonisins to milling fractions leading to increased concentrations in some and decreased concentrations in other products; and (iii) dissolution of fumonisins in aqueous cooking or steeping media which, if the liquid is discarded, reduces the fumonisins content of the product. It is now recognized that thermal processing (baking, frying, roasting, extruding) at temperatures above 150 – 200°C reduces the fumonisin concentrations that are measured in the cooked products. The chemical/physical basis of the decreases are poorly understood but probably reflect: (iv) chemical conversion of fumonisins to other compounds (for example, the hydrolyzed fumonisins formed during alkaline cooking); and (v) binding of fumonisins to sugars, proteins or other compounds present in the corn and other recipe ingredients. The degree to which fumonisin concentrations are reduced during cooking depends on temperature, cooking/processing time, pH, moisture content, and the recipe, especially the type and amount of sugar present.

It is important to emphasize that the measured fumonisin concentrations in cooked products might underestimate the products' potential toxicity due to the formation of unknown biologically active fumonisin degradation products or, as suggested by some recent findings concerning "hidden" fumonisins, reversible binding of the mycotoxin to sugar or proteins in the food matrix. Conversely, fumonisin degradation products might be less active or inactive, or fumonisins might bind to sugars or other compounds in a manner rendering them biologically inactive. While there is experimental evidence suggesting that most of these chemical reactions can occur during thermal processing, the issue of how these reactions affect fumonisin toxicity has only been superficially addressed. Additional investigations using an integrated approach combining chemical studies and appropriate bioassay methods are needed to identify and chemically characterize fumonisin reaction products: to determine the chemical fate of fumonisins under various cooking/processing conditions using a mass balance approach to account for all fumonisins in the starting materials, to identify those conditions that maximize fumonisin reductions and, to compare the biological activities of cooked products and their uncooked starting materials products using fumonisin-specific *in vitro* and *in vivo* bioassays.

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